

Reproductive biology of *Humerana miopus* (Anura: Ranidae) from Kedah, Peninsular Malaysia

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Humerana miopus is an intermediate to large-sized frog, with a snout-vent length reaching 73 mm (Berry, 1975). This forest species is distributed in southern Thailand and Peninsular Malaysia, and occurs at low altitudes (IUCN, 2014). It can often be encountered in primary rainforests, along logging tracts and in secondary growth forests. Additionally, it can be found along the river banks, forest edges, and overgrown rubber plantations, and is known to breed in pools (Grandison, 1972; Berry, 1975). It is the only species of frog from genus *Humerana* that existed in the forest of Peninsular Malaysia.

On 8 October 2017, between 21:30 and 22:30 h, an amplexic pair of *H. miopus* was collected at Sungai Sedim Recreational Forest, Kedah, Peninsular Malaysia (5°25'N, 100°46'E; elevation about 150 m a.s.l.). It was captured at the edge of a forest pool after heavy rainfall (2.5 hours rain). The temperature and relative humidity of the sampling site was 25 °C and 85%, respectively (Thermo Hydro meter, Fisher Scientific). The pool (approximately 3.5 m long, 3 m wide, and 0.02-0.4 m deep) was shaded and surrounded by low vegetation and was located along a trail within a lowland dipterocarp forest. The pool had a muddy bed and contained turbid water, with leaf litter, twigs, and tree branches accumulating at the bottom. In addition to the amplexic pair of *H. miopus*, five *H. miopus* (Fig. 1) and three *Fejervarya limnocharis* were detected at the same pool. This indicated that the pool served as a breeding site for both species of frogs.

The amplexic pair was captured and brought back to the laboratory for further inspections. Snout-vent length (SVL) and head width (HW) of male (SVL=68 mm, HW=19 mm) and female (SVL=77 mm, HW=23 mm) were measured by using digital calliper. Frogs were placed in a glass aquarium (60 x 30 x 30 cm), consisting of tap water (15 cm deep), sands, leaf litter, and drift woods. Frogs remained in amplexus, until the female deposited its eggs on 9 October 2017 (approximately 12 hours after captured). The eggs were sphere in shape, black in colour, coated by viscous jelly, and floating on the surface of water (Fig. 2). The clutch, which consisted 856 eggs were in a single layer. Twenty eggs were randomly selected and measured using a microscope with an ocular micrometer. The mean \pm SD (min-max, N) egg diameter was 1.1 ± 0.09 (0.9-1.2, 20) mm. After the study period, both frogs were released back to their original location.



Figure 1. An adult male of *H. miopus* from Kedah, Peninsular Malaysia

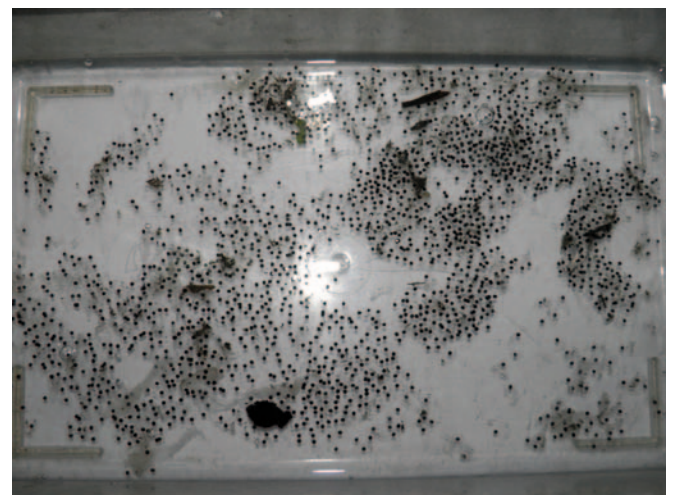


Figure 2. Egg clutch of *H. miopus*

The egg clutch was kept in the same aquarium until hatching (10 October 2017, approximately 28 hours post oviposition). An aerator was provided, to supply sufficient oxygen to the embryos. Only 472 (55%) of the eggs successfully hatched and became free-swimming tadpoles. Tadpoles were small, slender, and black in colour. Most of the tadpoles stayed at the bottom of the aquarium and only some at the surface of the water. At this stage, the mean \pm SD



Figure 3. Tadpole of *H. miopus*

(min-max, N) length of the tadpole was 4.9 ± 0.93 (3.0-6.0, 20) mm (Gosner's stage 19, Gosner 1960). Tadpoles were fed small fish pellets and rotten dead leaves. On 30 October 2017 (20 days post hatching), the mean \pm SD (min-max, N) total length of the tadpole was 18.9 ± 2.3 (16.0-23.0, 20) mm (Gosner's stage 25). The tadpole body was oval shaped and light to dark-brown in colour, with a light brown/yellow tapering tail, a white belly, and black spots on the body and tail (Fig. 3). Tadpoles had dorso-lateral located eyes, with pinkish colouration behind its eyes, and nostril at the end of a rounded snout. The tadpoles grazed on rotten leaves and spent most of its times at the bottom of the aquarium (usually under leaf litter). However, sometimes tadpoles would emerge to the surfaces of water to breathe. The order of larvae development was followed Gosner's stages (Gosner, 1960).

Previously, reproductive biology of several frog species from Peninsular Malaysia have been studied and documented. These included *Fejervarya limnocharis* and *F. cancrivora* (Ibrahim et al., 1999), *Chalcorana labialis* (Shahriza et al., 2010, 2016), *Ingerophrynus parvus* (Shahriza et al., 2012, 2015), *Sylvirana nigrovittata* (Shahriza, 2016) and *Rhacophorus prominanus* (Shahriza, 2017). This observation will increase the current knowledge and understanding of the ecology and breeding biology of tropical frogs.

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